

Vegetative Compatibility Groups in *Verticillium dahliae* Isolates from Cotton in Turkey

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Verticillium dahliae Kleb. with a complicated genetic diversity is a widely distributed major pathogen resulting in cotton wilt, which causes high economic losses in cotton lint production in the cotton belt of Turkey. A collection of 70 Turkish *V. dahliae* isolates (68 from wilted cotton plants in 28 districts and two from watermelon plants in two districts) were tested for vegetative compatibility by observing heterokaryon formation among complementary nitrate-nonutilizing (*nit*) mutants. The mutants were tested against international reference tester isolates and also were paired with one another. Thirty-nine isolates were assigned to vegetative compatibility group (VCG) 2B, 19 to VCG2A and three to VCG4B. One isolate was self-incompatible and eight others could not be assigned to any of the identified VCGs because their *nit* mutants showed negative reactions with the tester isolates of four VCGs or their *nit* mutants reverted back to the wild type. This is the first report of VCGs in *V. dahliae* from cotton in Turkey.

KEY WORDS: *Verticillium* wilt; *nit* mutants; VCGs; *Gossypium hirsutum*.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a widespread industrial crop grown over a large acreage in Turkey (2). Approximately 600,000–700,000 ha of cotton is grown annually in Turkey, most of it in the East Mediterranean Region with high summer temperatures, where cotton is grown intensively in fertile clayey soil. Short-term crop rotations and intensive cotton cultivation are the major results of high potential yield production and income from cotton farming as compared with the rest of the crops produced in the region. However, high cotton yield productivity potential of the cotton-growing soils is endangered by a disease called *Verticillium* wilt, caused by the soilborne fungal vascular-wilt pathogen *Verticillium dahliae* Kleb., and the disease has become a major constraint in the use of cotton as a crop in many fields in this region (15). In order to overcome or at least reduce the impact of the disease, cotton has recently been grown in rotation with crops such as corn and wheat, which greatly reduced *V. dahliae* inoculum density in the soil and weakened the *Verticillium* wilt epidemics (unpublished data). Nevertheless, *V. dahliae* is still the most important pathogen of cotton and causes economic losses in cotton lint yields in Turkey.

Fungal strains that anastomose and form viable heterokaryons with one another are considered to be vegetatively compatible and are assigned to a single vegetative compatibility grouping (VCG) (10). In *V. dahliae*, an anamorphic fungus with no known sexual stage, hyphal anastomosis and heterokaryosis are the only means of exchanging genetic

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information among different strains (13). VCGs as a means of subdividing the populations of several plant pathogenic and non-pathogenic fungi have proved to be a useful tool in characterizing genetic diversity among *V. dahliae* populations (1,6,7,11,13,14,16,18). Different VCGs have distinct pathogenicity, macroscopic and microscopic morphology, and temperature-dependent growth rates (13). Using complementary auxotrophic nitrate non-utilizing (*nit*) mutants, Joaquim and Rowe characterized isolates from *V. dahliae* into VCG 1-4 (10). Among isolates collected from a variety of plant species, most were classified into three main groups: VCG1, VCG2 (including subgroups 2A and 2B) and VCG4 (including 4A, 4B and 4AB) (6,10,17). The VCG diversity in Europe, including the Mediterranean area, is more limited than the one in the USA, where up to five VCGs with several subgroups have been reported (9). The objective of our study was to determine the VCGs diversity in Turkey and to analyze their relatedness to internationally recognized VCGs.

MATERIALS AND METHODS

Isolation and identification *Verticillium dahliae* was isolated from cotton plants showing Verticillium wilt symptoms, collected from *V. dahliae*-infested fields of southern Turkey in the summer seasons of 2000–2002 and transferred to the Mycology Laboratory, Department of Plant Protection of Cukurova University. Pieces of vascular tissue from basal stem segments were surface-sterilized with 0.5% sodium hypochlorite, rinsed in sterile distilled water, placed on potato-dextrose agar (PDA) amended with 100 mg l^{-1} streptomycin sulphate, and incubated for 7 days at 24°C until colonies with verticillately branched conidiophores formed around the plant tissue. Conidia from colonies were streaked on PDA plates and incubated at 24°C until small colonies grew on the medium. One isolate was obtained from each plant. Identification of *V. dahliae* was based on morphological characteristics (8). Monoconidial cultures were obtained from each *V. dahliae* isolate and maintained on PDA medium at 5°C.

Generation and characterization of *nit* mutants To select *nit* mutants, water agar-chlorate medium (WAC) was used. This medium was based on water agar (2%) with 0.02% glucose amended with 3–4% potassium chlorate (12). Mycelial plugs ($\sim 1 \text{ mm}^2$) cut from the edge of monoconidial cultures were placed on WAC in the center in 9 cm-petri plates, and incubated at 24°C in the dark. After 21 days of incubation, chlorate-resistant sectors, which appear as patches of dense aerial mycelium around the inoculum or scattered about the colony or as fan-like sectors at the colony perimeter, were transferred in 5-cm-diam petri dishes containing Czapek-Dox Agar (CDA), and allowed to grow for 5 days. Only sectors that grew on CDA as colonies with a thin, expansive mycelium were considered *nit* mutants. CDA amended with sodium nitrite (0.5 g l^{-1}) or hypoxanthine (0.2 g l^{-1}) was used for partial phenotyping of the *nit* mutants (3). Mutants that grew profusely (similar to wild type) on CDA with nitrite or hypoxanthine were classified as *nit1*. Mutants that grew sparsely on CDA with hypoxanthine were classified as NitM. Mutants that grew profusely on hypoxanthine and sparsely on nitrite were classified as *nit3*.

Complementation and vegetative compatibility This part of the work was carried out in three main steps. In the first part of the experiments, ten local tester isolates were randomly selected only from the isolates which produced the pairs of NitM / *nit1* phenotypes mutants. Mutants of local tester isolates were paired with complementary international tester isolates

(originating from Israel and USA) of previously described VCGs, and then these local testers were paired with one another among themselves. In the second part, *nit* mutants of 50 local isolates were paired with these local tester isolates. In the third part, the mutants of all isolates were also paired with complementary international tester isolates of previously described VCGs. International *nit* mutant testers of VCGs 1, 2A, 2B, 3, 4A, and 4B (Table 1) were kindly provided by N. Korolev and used to assign our isolates to previously characterized VCGs.

Complementation between phenotypically distinct *nit* mutants was tested on CDA. Generally, each 5-cm-diam petri dish was inoculated with three mutants, 1 cm apart in a triangular pattern, and incubated for 28 days at 24°C in the dark. Plates were scored for prototrophic growth 7–28 d after inoculation. Complementation was evident by the formation of a dense, aerial growth where mycelia from two mutants had met and formed a stable heterokaryon (3). When complementary mutants of two different isolates formed a heterokaryon, their parents were assigned to the same VCG. Each pairing was performed at least twice.

RESULTS

Collection of isolates The isolates originated from 52 sites at 30 locations in Turkey. In all, 34 isolates (Ch01-Ch34) were obtained from diseased cotton plants from 15 locations in Hatay province, 26 (Cc1-Cc5, Cka1-Cka6, Cko1-Cko8, Cy1-Cy7) from eight locations in Adana province, three (Co1-Co3) from two locations in Osmaniye province and three (Cm1-Cm3) from two locations in Kahramanmaraş province of southern Turkey (Table 2). The major cotton cultivars used at these locations were Cukurova 1518, SG 125 and DP 5690. All isolates except for two were recovered from the East Mediterranean Region of Turkey. Cig2 and Cig3 cotton isolates collected from Guzelkoy Region of western Turkey were the generous gift of Dr. M. Yildiz (Aegean University). Additionally, two *V. dahliae* isolates (W1-W2) obtained from diseased watermelon plants in Mersin province were used in the present study as local tester isolates. In all, 70 single-spore isolates of *V. dahliae* were obtained and used in VC studies.

TABLE 1. Origin, mutant phenotypes and vegetative compatibility groups (VCGs) of the tester isolates of *Verticillium dahliae* (refs. 10, 13)

Isolate	Origin of isolate	Mutant phenotype	VCG
cot228	Israel	<i>nit1</i>	1
T9	USA	NitM	1
ep52-1	Israel	<i>nit1</i>	2A
ep52-2	Israel	<i>nit1</i>	2A
ep8	Israel	NitM	2A
cot256	Israel	<i>nit1</i>	2B
cot11	Israel	NitM	2B
70-21	USA	NitM	3
171	USA	<i>nit1</i>	4A
131	USA	NitM	4A
R-5	USA	<i>nit1</i>	4B
Pt15	Israel	NitM	4B
Pt9G	Israel	<i>nit1</i>	4B

TABLE 2. Isolates of *Verticillium dahliae* from cotton and watermelon listed by geographic origin, their *nit1* mutants phenotypes and vegetative compatibility group (VCG)

Isolate	Host of origin	Geographic origin		Mutant phenotype	VCG
		Province	Location		
Cc3	Cotton	Adana	C. Korkuyu	<i>nit1</i>	2A
Cc5	Cotton	Adana	Ceyhan	<i>nit1</i>	2A
Ch02	Cotton	Hatay	Melekli	<i>nit1</i> and NitM	2A
Ch08	Cotton	Hatay	Paşaköy	<i>nit1</i>	2A
Ch10	Cotton	Hatay	Melekli	<i>nit1</i>	2A
Ch13	Cotton	Hatay	Melekli	<i>nit1</i>	2A
Ch14	Cotton	Hatay	Alaattin	<i>nit1</i>	2A
Ch15	Cotton	Hatay	Zulufluhan	<i>nit1</i>	2A
Ch16	Cotton	Hatay	Paşakoy	<i>nit1</i>	2A
Ch18	Cotton	Hatay	Melekli	<i>nit1</i>	2A
Cka1	Cotton	Adana	Karatas	<i>nit1</i> and NitM	2A
Cka2	Cotton	Adana	Karatas	<i>nit1</i> and NitM	2A
Cko4	Cotton	Adana	Imamoglu	<i>nit1</i>	2A
Cko7	Cotton	Adana	Imamoglu	<i>nit3</i> and <i>nit1</i>	2A
Co2	Cotton	Osmaniye	Degirmenocagi	<i>nit1</i>	2A
Cy1	Cotton	Adana	Yuregir	<i>nit1</i> and NitM	2A
Cy2	Cotton	Adana	Yuregir	<i>nit1</i>	2A
Cy3	Cotton	Adana	Yuregir	<i>nit1</i>	2A
Cy6	Cotton	Adana	Yuregir	<i>nit1</i>	2A
Cc1	Cotton	Adana	Ceyhan	<i>nit1</i> and NitM	2B
Cc4	Cotton	Adana	Ceyhan	<i>nit1</i>	2B
Ch01	Cotton	Hatay	Apaydin	<i>nit1</i> and NitM	2B
Ch03	Cotton	Hatay	Narlıca	<i>nit1</i> and NitM	2B
Ch04	Cotton	Hatay	Apaydin	<i>nit1</i> and NitM	2B
Ch05	Cotton	Hatay	Serinyol	<i>nit1</i>	2B
Ch06	Cotton	Hatay	Paşakoy	<i>nit1</i>	2B
Ch09	Cotton	Hatay	Hacıpasa	<i>nit1</i>	2B
Ch11	Cotton	Hatay	Narlıca	<i>nit1</i>	2B
Ch12	Cotton	Hatay	Apaydin	<i>nit1</i>	2B
Ch17	Cotton	Hatay	Topbogazı	<i>nit1</i>	2B
Ch19	Cotton	Hatay	Yesilova	<i>nit1</i>	2B
Ch20	Cotton	Hatay	Yesilova	<i>nit1</i>	2B
Ch22	Cotton	Hatay	Topbogazı	<i>nit1</i>	2B
Ch23	Cotton	Hatay	Topbogazı	<i>nit1</i>	2B
Ch24	Cotton	Hatay	Bohsin	<i>nit1</i>	2B
Ch25	Cotton	Hatay	Akpınar	<i>nit1</i>	2B
Ch26	Cotton	Hatay	Yesilova	<i>nit1</i>	2B
Ch27	Cotton	Hatay	Serinyol	<i>nit1</i>	2B
Ch28	Cotton	Hatay	Paşakoy	<i>nit1</i>	2B
Ch29	Cotton	Hatay	Demirköprü	<i>nit1</i>	2B
Ch31	Cotton	Hatay	Akkuyu	<i>nit1</i>	2B
Cka3	Cotton	Adana	Karatas	<i>nit1</i>	2B
Cka4	Cotton	Adana	K. Sirkenli	<i>nit1</i>	2B
Cka5	Cotton	Adana	Karatas	<i>nit1</i>	2B
Cka6	Cotton	Adana	K. Cakıroren	<i>nit1</i>	2B
Cko3	Cotton	Adana	Kozan	<i>nit1</i>	2B
Cko5	Cotton	Adana	Imamoglu	<i>nit1</i>	2B
Cko6	Cotton	Adana	Imamoglu	<i>nit1</i>	2B
Cm1	Cotton	K. Maras	Sereflioglu	<i>nit1</i> and NitM	2B
Cm2	Cotton	K. Maras	Narliovasi	<i>nit1</i>	2B
Cm3	Cotton	K. Maras	Sereflioglu	<i>nit1</i>	2B
Co1	Cotton	Osmaniye	Aslaniye	<i>nit1</i> and NitM	2B
Co3	Cotton	Osmaniye	Degirmenocagi	<i>nit1</i>	2B

TABLE 2 (cont'd.)

Isolate	Host of origin	Geographic origin		Mutant phenotype	VCG
		Province	Location		
Cy5	Cotton	Adana	Yuregir	<i>nit1</i>	2B
Cy7	Cotton	Adana	Yuregir	<i>nit1</i>	2B
Cig3	Cotton	Izmir	Guzelkoy	<i>nit1</i>	2B
W1	Watermelon	Mersin	Tarsus	<i>nit1</i> and NitM	2B
W2	Watermelon	Mersin	Yenice	<i>nit1</i> and NitM	2B
Ch21	Cotton	Hatay	Serinyol	<i>nit1</i>	4B
Cko1	Cotton	Adana	Kozan	<i>nit1</i> and NitM	4B
Cy4	Cotton	Adana	Yuregir	<i>nit3</i> and <i>nit1</i>	4B
Ch07	Cotton	Hatay	Reyhanlı	<i>nit3</i> and <i>nit1</i>	HSI
Ch32	Cotton	Hatay	Alaattin	<i>nit1</i>	NC**
Ch33	Cotton	Hatay	Serinyol	<i>nit1</i>	NC**
Cko2	Cotton	Adana	Imamoglu	<i>nit1</i> and NitM	NC**
Cko8	Cotton	Adana	Imamoglu	<i>nit1</i>	NC**

HSI, Heterokaryon self-incompatible.

NC=Not characterized.

* All *nit* mutants obtained from these isolates showed negative reactions with the local and international tester isolates of four VCGs.

Generation and characterization of *nit* mutants Chlorate-resistant sectors appeared infrequently but their visual distinction was certain. In 10 to 15 replications, each of 70 isolates of *V. dahliae* produced 5 to 20 chlorate-resistant sectors, 3 to 7 of which were phenotyped as *nit* mutants and not less than one *nit* mutant was recovered for each isolate of *V. dahliae*. Some mutants had a tendency to revert back to the wild type. Mutants from the isolates were usually characterized within 10 days after collecting them from the chlorate-amended medium. Most mutants grew profusely (similar to wild type) on CDA with nitrite or hypoxanthine and were classified as *nit* (78.6%). Some (16.6%) of the *nit* mutants grew sparsely on CDA with hypoxanthine and were classified as NitM. Four mutants grew profusely on hypoxanthine and sparsely on nitrite medium and were classified as *nit* mutants. While some of the *nit* mutants grew profusely on CDA with hypoxanthine and did not grow on nitrite medium, their phenotype was not clear. Nevertheless, such mutants of *V. dahliae* were phenotyped as *nit1*. All *nit* mutants showed wild-type growth on PDA.

Complementation and vegetative compatibility To determine VCGs during the first stage of the research study, complementation tests were carried out among *nit1* and NitM mutants generated from ten isolates (Cm1, Cc1, W1, Ch01, Co1, W2, Cy1, Ch02, Cka1, Cko1) chosen as VCG local testers (Table 3). Table 3 shows the compatibility reactions between complementary *nit* mutants of international testers and local Turkish testers. First six local tester isolates shown in the table were assigned to VCG2B and these isolates showed strong reactions with the *nit* mutants from the VCG2B testers cot256 and cot11 while these were incompatible with the other VCGs mutants. Among the pairings with VCG2B local testers, the weak reactions were observed for W1 and W2 with *nit* mutants from 171 and 131, Co1 with *nit* mutants from 171, and Cc1 with *nit* mutants from 131 of VCG4A. The other *nit* mutants from Cy1, Ch02 and Cka1 isolates showed strong compatibility with at least two of the VCG2A testers ep52-2, ep8 and ep52-1, but not with the other VCG testers. Of these three isolates, Cy1 reacted weakly with *nit* mutants

from cot256 of VCG2B, R-5 of VCG4B and with all VCG4A *nit* mutants. The isolate was incompatible with Pt15 and pt9G of VCG4B, and with all *nit* mutants of VCG1 and VCG3. Consequently, these three were assigned to VCG2A. Finally, the only strongly reacting isolate with international testers of VCG4B was Cko1. VCG4B local tester Cko1 reacted negatively with all testers of VCGs 2B, 1, 3 and with VCG2A tester ep52-2, whereas ep8 and ep52-1 testers of VCG2A reacted weakly with it. Overall, local tester isolates were classified as belonging to VCGs 2B (Cm1, Cc1, W1, Ch01, Co1, W2), 2A (Cy1, Ch02, Cka1) and 4B (Cko1) (Table 3). International testers of VCG1 and VCG3 showed no reaction when paired with these local testers.

TABLE 3. Complementation between complementary *nit* mutants of international tester isolates (horizontal line) and local tester isolates (vertical line) of *Verticillium dahliae*

Local testers	VCG2B		VCG2A			VCG4A		VCG4B			VCG1	VCG3
	cot256	cot11	ep52-2	ep8	ep52-1	171	131	R-5	Pt15	Pt9G	cot228T9	70-21
Cm1	+	+	-	-	-	-	-	-	-	-	-	-
Cc1	+	+	-	-	-	-	±	-	-	-	-	-
W1	+	+	-	-	-	±	±	-	-	-	-	-
Ch01	+	+	-	-	-	-	-	-	-	-	-	-
Co1	+	+	-	-	-	±	-	-	-	-	-	-
W2	+	+	-	-	-	±	±	-	-	-	-	-
Cy1	±	-	+	+	+	±	±	±	-	-	-	-
Ch02	-	-	+	-	+	-	-	-	-	-	-	-
Cka1	-	-	-	+	-	-	-	-	-	-	-	-
Cko1	-	-	-	±	±	-	±	+	+	+	-	-

+, Dense prototrophic growth; -, no prototrophic growth; ±, weak reaction.

TABLE 4. Complementation between different mutant phenotypes of ten local *Verticillium dahliae* tester isolates from Turkey

Local testers	VCG	Cm1	Cc1	W1	Ch01	Co1	W2	Cy1	Ch02	Cka1	Cko1
	2B	2B	2B	2B	2B	2B	2B	2A	2A	2A	4B
Cm1	2B	+									
Cc1	2B	+	+								
W1	2B	+	+	+							
Ch01	2B	+	+	+	+						
Co1	2B	+	+	+	+	+					
W2	2B	+	+	+	+	+	+				
Cy1	2A	-	-	-	-	-	-	+			
Ch02	2A	-	-	-	-	-	-	+	+		
Cka1	2A	-	-	-	-	-	-	+	+	+	
Cko1	4B	±	-	-	-	-	-	-	±	±	+

+, Dense prototrophic growth; -, no prototrophic growth; ±, weak reaction.

Table 4 shows the complementation between mutants of ten different Turkish-origin tester isolates of *V. dahliae*. According to this tabulation, the compatibility reactions between the mutants from the first six different local isolates of VCG2B were positive. The pairings of testers Cy1, Ch02 and Cka1 of VCG2A with the former six isolates of VCG2B proved negative compatibility. Further, the negatively paired isolates of different subgroups displayed a pronounced positive complementation in their own subgroups. Nevertheless, Cko1 (VCG4B) was weakly compatible with the VCG2A testers Ch02 and Cka1 and the

VCG2B tester Cm1 (Table 4). Different *nit* phenotypes derived from the same isolates were compatible with each other, showing that parental isolates are self-compatible.

In the second part of the complementation works, the *nit* mutants of 50 *V. dahliae* isolates from the cotton belt of Turkish agriculture were tested against complementary mutants of these local testers (*nit1* x *nit3*, *nit1* x NitM, *nit3* x NitM) (Table 5). Heterokaryon formation appeared as a dense aerial wild-type mycelium where two complementary mutants had met. The most robust heterokaryosis was observed in pairings between NitM and *nit1* mutants. A weak complementation sometimes occurred also between different *nit1* and *nitM* mutants. The formation of heterokaryons was first seen 7 days after pairing and the observations were continued up to 28 days. Three distinct groups were observed during this step of complementation studies. These groups were correlated with previously characterized VC subgroups 2A, 2B and 4B. All isolates in VC subgroups 2A and 2B complemented strongly with at least two local testers of these subgroups. Two isolates (Ch21 and Cy4) belonging to VCG4 reacted strongly with the Cko1 tester (VCG4B). However, local tester isolate Cko1 complemented weakly with the most isolates of VCG2A and with some isolates belonging to VCG2B. Additionally, the Ch10 isolate (VCG2A) complemented strongly with all local VCG2A testers (Cy1, Ch02 and Cka1) but also with the Cc1 and Ch01 of VCG2B.

In the final step of complementation processes, to assign and compare the VCGs in Turkey with those previously described, representative mutants of the local isolates were paired with international tester isolates (Table 6). Fifty-nine percent of the Turkish isolates registered very strong positive pairings with the VCG2B international *nit* mutant cot11, while 41% of the local isolates yielded a negative reaction with cot11 (Table 6). This result may mean the *nit* mutant cot11 is a very common partner of the Turkish isolates. The international *nit* mutant cot256 was not tested with all local isolates and the pairings of the local isolates with this alien mutant generally produced negative reactions. Table 6 shows the second major partner for the Turkish isolates is likely to be the VCG2A's international *nit* mutant ep8, which is mostly in negative relations with the Turkish isolates (68%), but also in a reasonable amount of weak (3%) and positive (29%) reactions with some Turkish isolates. For the VCG4B, Cy4 and Ch21 local isolates are in a positive relationship with the alien VCG4B mutants. Of all VCGs, VCG4 testers provided the largest amount of weak reactions with the local isolates (Table 6). Overall, based on positive complementation reactions with the complementary tester isolates' mutants, two multimember VCGs (VCG2 and VCG4) were identified among the 66 isolates (Table 3 and 6). Of these two VCGs, three multimember VCG subgroups (VCG2A, VCG2B, VCG4B) were identified. However, nine isolates (13.2%) could not be assigned to any of these two VCGs. The *nit* mutants obtained from five isolates (Ch07, Ch32, Ch33, Cko2 and Cko8) showed negative reactions with the local and international tester isolates of four VCGs. Among these isolates, Ch32, Ch33 and Cko8 did not produce two different *nit* mutant phenotypes and therefore could not be tested for self-compatibility. While Cko2 was found to be self-compatible, Ch07 was self-incompatible. Although the pairings with isolates Ch32, Ch33, Cko2 and Cko8 were repeated five times, these four isolates were not found compatible with the local tester isolates and international tester isolates of four VCGs. Consequently, these four isolates may represent new VCGs. VC belonging to four other isolates (Cc2, Ch30, Ch34 and Cig2) was not determined, because all mutants obtained from these isolates reverted back to the wild type.

TABLE 5. Complementation between NitM mutants of local tester isolates (horizontal line) and *nit1* or *nit3* (vertical line) mutants of 50 *Verticillium dahliae* isolates

	2B						2A			4B
	Cm1	Cc1	W1	Ch01	Co1	W2	Cy1	Ch02	Cka1	Cko1
Cc4	-	+	+	+	+	+	-	-	-	±
Ch05	+	+	+	+	+	+	-	-	-	-
Ch06	+	+	+	+	±	+	-	-	-	-
Ch09	-	+	+	+	+	+	-	-	-	±
Ch11	-	+	+	+	+	+	-	-	-	±
Ch12	-	+	-	-	+	+	-	-	-	±
Ch17	-	+	-	-	+	+	-	-	-	±
Ch19	±	+	+	+	+	+	-	-	-	-
Ch20	-	+	+	+	+	+	-	-	-	-
Ch22	-	+	+	+	-	+	-	-	-	-
Ch23	-	+	+	+	-	+	-	±	-	-
Ch24	-	+	+	+	-	+	-	-	-	-
Ch25	-	+	+	+	±	+	-	+	-	-
Ch26	+	+	+	+	-	-	-	-	-	-
Ch27	-	+	+	+	+	+	-	-	-	-
Ch29	-	+	+	+	-	+	-	-	-	-
Ch31	-	+	+	+	-	+	-	-	-	-
Cka3	-	+	+	-	+	+	-	-	-	-
Cka4	-	+	+	-	+	+	-	-	-	±
Cka5	-	+	+	+	-	+	-	-	-	-
Cka6	-	+	+	-	+	+	-	-	-	-
Cko3	-	+	+	-	+	+	-	-	-	-
Cko5	-	+	+	-	+	+	-	-	-	-
Cko6	-	+	+	-	+	+	-	-	-	-
Cm2	+	+	+	+	+	+	-	-	-	±
Cm3	+	+	+	+	+	+	-	-	-	±
Co3	-	+	+	-	+	+	-	-	-	-
Cy5	-	+	+	+	+	+	-	-	-	-
Cy7	-	+	+	+	+	+	-	-	-	-
Cig3a	-	+	+	+	-	+	-	-	-	-
Cc3	-	-	-	-	-	-	+	+	+	-
Cc5	-	-	-	-	-	-	+	+	+	-
Ch08	-	-	-	-	-	-	+	+	+	±
Ch10	-	±	+	-	±	+	+	+	+	±
Ch13	-	-	-	-	-	-	+	-	+	±
Ch14	-	-	-	-	-	-	+	+	+	±
Ch15	-	-	-	-	-	-	+	+	+	±
Ch16	-	-	-	-	-	-	+	-	+	±
Ch18	-	-	-	-	-	-	+	+	+	-
Cko4	-	-	-	-	-	-	+	+	+	±
Co2	-	-	-	-	-	-	-	-	-	±
Cy2	-	-	-	-	-	-	+	+	+	±
Cy3	-	-	-	-	-	-	+	+	+	-
Cy6	-	-	-	-	-	-	+	+	+	-
Ch21	-	-	-	-	-	-	-	-	-	+
Cy4	-	-	-	-	-	-	-	-	-	+
Ch07	-	±	-	-	-	-	-	-	-	-
Ch32	-	-	-	-	-	-	-	-	-	-
Ch33	-	-	-	-	-	-	-	-	-	-
Cko8	-	-	-	-	-	-	-	-	-	-

+, Dense prototrophic growth; -, no prototrophic growth; ±, weak reaction.

TABLE 6. Complementation between complementary *nit* mutants of international tester isolates (horizontal line) and Turkish isolates (vertical line) of *Verticillium dahliae*

Turkish isolates	VCG2B		VCG2A			VCG4A		VCG4B			VCG1		VCG3
	cot256	cot11	ep52-2	ep8	ep52-1	171	131	R-5	Pt15	Pt9G	cot228	T9	70-21
Cc4		+		-			-		-			-	-
Ch03	±	+	-	-	-	-	-	-	-	-	-	-	-
Ch04	+	+	-	-	-	-	-	-	-	-	-	-	-
Ch05		+		-			-		-			-	-
Ch06		+		-			±		-			-	-
Ch09		+		±			-		-			-	-
Ch11		+		-			±		-			-	-
Ch12		+		-			-		-			-	-
Ch17		+		-			-		-			-	-
Ch19		+		-			-		-			-	-
Ch20		+		-			-		-			-	-
Ch22		+		±			-		-			-	-
Ch23		+		-			-		-			-	-
Ch24		+		-			±		-			-	-
Ch25		+		-			-		-			-	-
Ch26		+		-			-		-			-	-
Ch27		+		-			-		-			-	-
Ch28		+		-			-		-			-	-
Ch29		+		-			-		-			-	-
Ch31		+		-			-		-			-	-
Cka3		+		-			-		-			-	-
Cka4		+		-			±		-			-	-
Cka5		+		-			-		-			-	-
Cka6		+		-			-		-			-	-
Cko3		+		-			-		-			-	-
Cko5		+		-			-		-			-	-
Cko6		+		-			-		-			-	-
Cm2		+		-			-		-			-	-
Cm3		+		-			-		-			-	-
Co3		+		-			-		-			-	-
Cy5		+		-			-		-			-	-
Cy7		+		-			-		-			-	-
Cig3a		+		-			-		-			-	-
Cc3		-		+			-		-			-	-
Cc5		-		+			-		-			-	-
Ch08		-		+			-		-			-	-
Ch10		-		+			-		-			-	-
Ch13		-		+			-		-			-	-
Ch14		-		+			-		-			-	-
Ch15		-		+			-		-			-	-
Ch16		-		+			-		-			-	-
Ch18		-		+			-		-			-	-
Cka2		-	-	+	-	-	-	-	-	-	-	-	-
Cko4		-		+			-		-			-	-
Cko7		-	+	+	+	±	-	±	-	-	-	-	-
Co2		-		+			-		-			-	-
Cy2		-		+			-		-			-	-
Cy3		-		+			-		-			-	-
Cy6		-		+			±		-			-	-
Ch21		-		-			-		+			-	-
Cy4	-	-	-	-	-	-	-	+	+	+	-	-	-
Ch07	-	-	-	-	-	-	-	-	-	-	-	-	-
Ch32	-	-	-	-	-	-	-	-	-	-	-	-	-
Ch33	-	+	-	-	-	-	-	-	-	-	-	-	-
Cko2	-	-	-	-	-	-	-	-	-	-	-	-	-
Cko8	-	-	-	-	-	-	-	-	-	-	-	-	-

+, Dense prototrophic growth; -, no prototrophic growth; ±, weak reaction; blank space, no data.

VCG2 was the largest group and included 58 isolates, 82.9% of all isolates. VCG2A and VCG2B included 19 and 39 isolates, 32.8% and 67.2% of VCG2 isolates, respectively. VCG4B included three isolates, 4.3% of all isolates. VCG1 and VCG3 were not defined among the isolates tested, because all the isolates failed to anastomose with the VCG1 testers from T-9 and cot228 and also with VCG3 tester from the isolate 70-21.

DISCUSSION

Our data demonstrated that wilt of cotton in southern Turkey is caused mostly by *V. dahliae*, as indicated in a previous study by Bicici and Kurt (2). In the present study, vegetative compatibility of 70 *V. dahliae* isolates from Turkey was assessed using *nit* mutants. Some isolates produced *nit* mutants readily, whereas others produced a few mutants. *nit3* mutants were rarely produced in this study and the vast majority of mutants were of the *nit1* phenotype mutants. Some *nit3* mutants could not be distinguished from *nit1* because they did not grow on nitrite medium. Similar results were also reported by several researchers (12,13). In these studies, such mutants in *V. dahliae* were phenotyped as *nit1*, because they complemented NitM mutants but did not complement *nit1*; no *nit3* mutants were recognized among approximately 2000 *nit* mutants. Therefore, we also considered this kind of mutant as *nit1*.

Complementation between *nit* mutants of *V. dahliae* was complex because not all isolates within a VCG complement one another. Local tester NitM mutant from Cm1 isolate (VCG2B) was capable of complementing strongly with *nit* mutants from international reference isolates and local tester isolates of VCG2B (Tables 3 and 4), but *nit* mutants from local *Verticillium* isolates of VCG2B were mostly incompatible with it (Table 5). Furthermore, some weak complementation reactions were observed in pairings among isolates of different VCGs. For example, the mutants from Cko1 isolate (VCG4B) formed weakly compatible heterokaryons with the VCG2 local and international isolates (Tables 3, 4 and 5). The pair of *nit* mutants of the isolate Cko1 comprised NitM/ *nit1*, but this isolate appeared to be a so-called bridge isolate, as it made heterokaryons not only with VCG4B but also with VCG2 isolates. In the future, the chosen local tester Cko1 will be replaced by Cy4 mutants as a Turkish tester isolate of VCG4B, as the second one did not produce a cross-reaction, despite the fact that this pair lacks NitM and consists of *nit3*/*nit1* mutants. However, we made a good choice of testers of VCG2, getting clear-cut results in VCG grouping of VCG2A and VCG2B; there were very few weaker reactions between the isolates included in VCG2A with VCG2B (Tables 4 and 5). All the isolates failed to anastomose with the VCG1 and VCG3 testers (Tables 3 and 6).

This is the first study of vegetative compatibility of *V. dahliae* isolates from cotton in Turkey. Based on complementarity of *nit* mutants, we identified only two VCGs from Turkish isolates from cotton: VCGs 2 and 4. The study demonstrated that 58 isolates (~95.1% of the characterized isolates and 82.9% of the total sample) were assigned to VCG2 and three were assigned to VCG4. Besides, the number of VCG2B isolates is more than twofold that of VCG2A isolates (39 and 19 of VCG2 isolates, respectively). In the present study, the subgroup 2B of VCG2 is not very strongly connected to subgroup 2A of the same VCG, since the local testers Cy1, Ch02 and Cka1 of VCG2A were not even weakly compatible with the local testers of VCG 2B (Table 4). This negative incompatibility may mean VCG2A and VCG2B mutants have been acting in ways so different that their virulence on cotton species may require different plant protection

strategies, or they may be different VCGs. However, VCG2 was still the most common internationally detected and reported VCG in cotton and this was also confirmed in our studies. In Greece, a neighboring country of Turkey, 46 out of 71 Greek isolates of *V. dahliae* obtained from cotton were assigned to VCG2, two to VCG4 and one to VCG1. However, the remaining 22 isolates could not be assigned to any of the identified VCGs (5). In Israel, a Mediterranean country like Turkey, 135 of 201 Israeli isolates of *V. dahliae* obtained from cotton were assigned to VCG2B, one to VCG2A and 65 to VCG4B (13). The prevalence of VCG2 in the East Mediterranean Region indicates that *V. dahliae* population in this region is similar genetically to the isolates of the pathogen in Israel and Greece (6,13).

VCGs of the isolates did not differ in their geographic distribution in our region. Three VCG subgroups were recovered from Adana province, three from Hatay province, two from Osmaniye province, and one from Mersin province. In contrast to the findings of Daayf *et al.* (4), no correlation was apparent between VCG and site of isolation. For example, three isolates of VCG4B were obtained from three different provinces (Serinyol-Hatay, Kozan-Adana and Yuregir-Adana). Additionally, isolates obtained from the same locations often belonged to more than one VCG (Table 2). The data presented in this study demonstrated that different VCGs were recovered from the same location; and the same VCG could be isolated from different sites (6). However, further studies are required to investigate more isolates from various hosts and geographic locations of Turkey in order to reveal VCG diversity in detail.

Our study presents the first results on vegetative diversity in *V. dahliae* from cotton in Turkey. Being the first study of the genetic diversity of *V. dahliae*, our work revealed quite low diversity in our population, since two different vegetative compatibility groups: VCG2 (with subgroups VCG2A and VCG2B) and VCG4 (with subgroup VCG4B) were found among 70 isolates obtained mostly from southern Turkey. However, this limited VCG diversity observed in Turkish cotton-growing lands corresponds well with the reports from other European countries, as summarized by Hiemstra and Rataj-Guranowska (9). Further work is needed to map the geographical spread of VCGs in *V. dahliae* from different hosts and to investigate the relationship between VCG and host specificity or virulence of individual isolates.

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